In fact, we have recently produced T*-like phage by growth on two *gal*$^-$ mutants of *E. coli* K-12 (isolated by Miss June Rothman), which are unable to utilize glucose as a carbon source but have normal levels of hexokinase, phosphoglucomutase, and UDPG-PPase. The genetic defect in these strains is not in the *gal* operon and probably concerns the ability to synthesize endogenous glucose-6-phosphate.

Summary.—Growth of the phages T2 and T4 in *E. coli* mutant strains defective in the enzyme UDPG-pyrophosphorylase yields phage particles of the T* type, whose DNA contains only about 10 per cent as much glucose as that of normal phage. T* phages fail to multiply in their normal *E. coli* hosts, but can reproduce in *S. dysenteriae* strain Sh.

The authors are grateful to Dr. S. E. Luria for suggestions and encouragement during the course of these experiments and for help in preparing the manuscript.

* Aided by grants from the National Science Foundation, G-8808, and the National Institute of Allergy and Infectious Diseases, National Institutes of Health, E3038 (C2).

† Trainee under training grant 2G-602 of the USPHS to the Department of Biology, Massachusetts Institute of Technology.

[1] Luria, S. E., in *Viruses*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 18 (1953), p. 237.

[2] Arber, W., and D. Dussoix, *J. Mol. Biol.*, **5**, 18 (1962).

[3] Luria, S. E., and M. L. Human, *J. Bacteriol.*, **64**, 557 (1952).

[4] Eriksen, R. L., and W. Szybalski, *Abstracts Biophysical Society 7th Annual Meeting* (1963), p. WA8.

[5] Shedlovsky, A., and S. Brenner, these PROCEEDINGS, **50**, 300 (1963).

[6] Fukasawa, T., K. Jokura, and K. Kurahashi, *Biochem. Biophys. Res. Comm.*, **7**, 121 (1962).

[6a] Sundararajan, T. A., A.M.C. Rapin, and H. M. Kalckar, these PROCEEDINGS, **48**, 2187 (1962).

[7] Fukasawa, T., K. Jokura, and K. Kurahashi, *Biochem. et Biophys. Acta*, in press.

[8] Munch-Petersen, A., H. M. Kalckar, E. Cutolo, and E. E. B. Smith, *Nature*, **172**, 1036 (1953).

[9] Marinetti, J., *Lipid Research*, **3**, 1 (1962).

[10] Fukasawa, T., and S. Saito, *Fed. Proc.*, **22**, 406 (1963).

A CHEMICAL BASIS FOR THE HOST-INDUCED MODIFICATION OF T-EVEN BACTERIOPHAGES

BY A. SHEDLOVSKY* AND S. BRENNER

MRC LABORATORY OF MOLECULAR BIOLOGY, CAMBRIDGE, ENGLAND

Communicated by S. E. Luria, June 26, 1963

Bacterial viruses may exist in different forms which are genetically indistinguishable but differ with respect to their ability to multiply in certain host bacteria. The unrestricted form can be propagated on a "permissive" host strain, on which the restricted form is unable to multiply. The conversion of virus particles from one state to the other in the course of a single cycle of growth has been termed host-induced modification. This phenomenon has been studied in a variety of bacterial virus systems[1-3], and it has recently been demonstrated that the host-induced modification of phage λ affects the DNA of the virus.[4, 5]

The experiments presented here reveal the chemical nature of a host-induced modification of T-even bacteriophage.

Materials and Methods.—Bacterial strains: Escherichia coli strain B and its derivative BB (Berkeley), and the mutants B/2, B/4, S/6, resistant to T2, T4, and T6, respectively. Strain HfrC *gal*$^-$ (U95) was isolated as a mutant of HfrC, a derivative of *E. coli* K-12, and is unable to utilize galactose as a sole carbon source. Although it has not been analyzed by enzyme assays, this mutant appears to have a defect in the synthesis of uridine diphosphoglucose (UDPG). In several respects it resembles mutant W4597 described by Fukasawa *et al.*[6] : galactose inhibits growth on glycerol, an observation suggesting that the mutant is unable to utilize galactose-1-phosphate.[7] The mutation in U95 is not located in any of the *gal* genes, as shown by the failure of F*gal* and λ*dg* to convert the strain to *gal.*$^+$ In addition, strain U95 does not contain galactose in its cell wall, as shown by its sensitivity to phage C21, a property shared by UDPGal-4-epimeraseless mutants.[8] The mutation in strain U95 appears to be located on the *streptomycin-TL* segment of the chromosome.

Phage strains: T2H, T4Br^+, T6c_2.

Media: M9 contains, per liter: 5.8 gm Na_2HPO_4, 3.0 gm KH_2PO_4, 0.5 gm NaCl, 1.0 gm NH_4Cl, 10^{-3} *M* $MgSO_4$, and 2 gm glucose. Broth contains, per liter: 10 gm Bacto tryptone and 5 gm NaCl.

Methods: Phage titers were measured using the agar layer technique[9] on broth agar plates. Purified phage stocks were prepared by infecting logarithmically growing bacteria (3–4 × 10^8/ml) with phage at a multiplicity of 3. After 6 min, the cells were superinfected with the same multiplicity, and incubation was continued for 4 hr. The cultures were then lysed with chloroform, and the phage was purified by two cycles of differential centrifugation. After the first cycle, the phage suspension was treated with RNAase and DNAase (5 μg/ml each). Radioactive phage stocks were prepared in the same way except that the bacteria were infected in M9 medium containing 10 μg/ml H^3-hypoxanthine (57 mc/mmole, Radiochemical Centre, Amersham).

Glucosylation of phage DNA: Glucosyl transferases, prepared according to the procedure of Kornberg *et al.*,[10] were not purified beyond the streptomycin precipitation step. UDPGal-4-epimerase was obtained as a crude extract of galactose-grown *E. coli* B. Phage DNA was prepared by shaking a purified phage suspension with an equal volume of freshly distilled, water-saturated phenol at room temperature for 2 min. The DNA was precipitated from the aqueous layer with two volumes of 95 % ethanol, collected on a glass stirring rod, and dissolved in 0.01 *M* NaCl in 0.01 *M* Tris buffer pH 7.6. After adjusting the salt concentration to 0.1 *M* NaCl, the DNA was reprecipitated and redissolved as above. Traces of of phenol were removed by repeated ether extraction, and the ether was eliminated by gently bubbling air through the DNA solution. It was found that these DNA preparations contained 180 absorbancy units (at 260 mμ) per mg phosphorus, as measured by the procedure of Allen.[11] UDPGal-C^{14} was prepared by a modification[12] of the procedure of Wiesmeyer and Jordan.[13]

Using the T4 β-glucosyl transferase, each ml of reaction mixture contained: UDPGal-C^{14}, 48 mμmoles; KPO_4 pH 7.8, 100 μmoles; $MgCl_2$, 25 μmoles; DNA, 10–20 μg; UDPGal-4-epimerase, 500 μg protein; glucosyl transferase, 0.2–0.3 ml. With the T4 α- or the T6 glucosyl transferase, each ml of reaction mixture contained: UDPGal-C^{14}, 48 mμmoles; Tris pH 7.8, 100 μmoles; mercaptoenthanol, 33

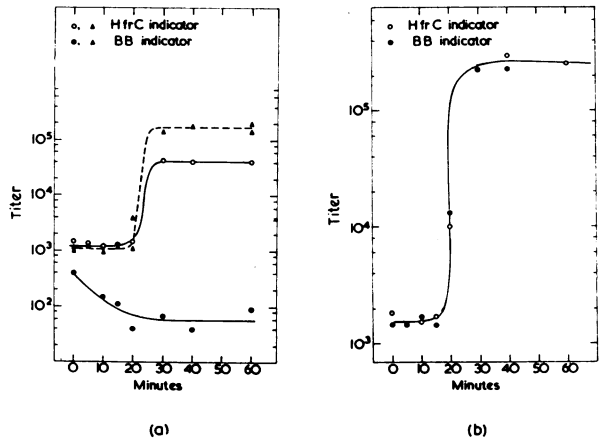

FIG. 1*a*.—One-step growth curve of T6·HfrC on strains HfrC and U95. Broth cultures of strains HfrC (triangles) and U95 (circles) in the exponential phase of growth (2×10^8/ml) were centrifuged. The bacteria were resuspended in buffer (M9 medium without glucose) and starved for 15 min at 37°C. L-tryptophan 10 μg/ml was added, and the cultures were infected with T6·HfrC at a multiplicity of 0.1, as determined by previous titration of the phage on HfrC indicator. After six min of incubation to allow adsorption, the cultures were diluted 10^{-4} into broth. During the subsequent incubation, samples were withdrawn and assayed for the number of infective centers on strains HfrC and BB.

FIG. 1*b*.—One-step growth curve of T6·U95 on strain HfrC. Experimental procedure as in Fig. 1*a*.

μmoles; ammonium sulfate, 15 μmoles; DNA 10–20 μg; UDPGal-4-epimerase, 500 μg protein; glucosyl transferase, 0.2–0.3 ml. The reaction was initiated by addition of glucosyl transferase. Samples of 0.2 ml were withdrawn and pipetted into 2 ml cold 5% TCA after incubation at 30°C. The samples, after standing in the cold for 10 min, were filtered onto membrane filters, which were washed twice with cold 5% TCA, glued to planchettes, air-dried, and used for radioactivity determination.

Results.--Host-induced modification of T6 by strain U95: Initial attempts to grow bacteriophage T6 on strain U95 led to apparently conflicting results. A stock of T6, prepared on *E. coli* strain B, contained 1.4×10^{11} plaque-forming units per ml when assayed either on strain B or on strain U95. However, when strain U95 was infected with this phage at a multiplicity of 0.1, no increase in titer, using B as indicator, was detected after 40 min of incubation. Thus, it appeared that T6 could produce plaques when plated on strain U95, but that the phage released from this mutant was unable to grow on strain B.

A comparison of the efficiency of plating (EOP) of purified stocks prepared on strain U95 (T6·U95) and on its parent strain HfrC (T6·HfrC) was made using a

TABLE 1

PLAQUE-FORMING UNITS PER ABSORBANCY 1.0 OF PURIFIED PHAGE STOCKS

Phage	BB	B	B/2	HfrC	U95
T6·HfrC	2.5×10^{10}	3×10^{10}	3.3×10^{10}	3.1×10^{10}	2.3×10^{10}
T6·U95	1.3×10^{7}	1.1×10^{7}	1.2×10^{7}	3.5×10^{9}	3.3×10^{9}

Overnight broth cultures of each bacterial strain were diluted into fresh medium and incubated for 3–4 generations until a cell density of 4×10^8/ml had been reached. Purified phage stocks were diluted and the titers determined on the different indicator strains. The absorbancy at 260 mμ of each phage stock was measured, and the numbers of plaque-forming units are expressed as titer per unit absorbancy.

variety of plating bacteria. Table 1 demonstrates that, per unit of absorbancy at 260 mμ (a measure of concentration of virus particles), T6·U95 has 0.05–10 per cent EOP of T6·HfrC. More important, it can be seen that T6·U95 has a very low EOP on strains derived from *E. coli* B.

The properties of these phages were examined in the course of a single cycle of growth of U95 and HfrC. It was found (Fig. 1*a*) that one cycle of growth in strain U95 modifies T6·HfrC with respect to its ability to form plaques on strain B, converting it to T6·U95. Furthermore, this modification is lost during a single passage through strain HfrC (Fig. 1*b*). Thus, the properties of these phages resemble in some respects those of the original T* host-induced modification of Luria and Human.[1] The data presented in Figure 1 demonstrate that the rate of growth of these phages is not affected by the host. Although the size of the burst from strain U95, as measured on HfrC indicator, appears to be only one tenth that obtained from strain HfrC, the number of virus particles produced is probably the same in both cases, since purified T6·U95 has an EOP per unit absorbancy on HfrC indicator one tenth that of T6·HfrC.

Bacteriophage T2 and T4 have also been grown on strain U95. The progeny, T2·U95 and T4·U95, exhibit properties similar to those just described for T6·U95. In the case of T2, however, the EOP is difficult to determine on derivatives of *E. coli* K-12, in which even the unmodified phage grow poorly; T4·U95 and T4·HfrC, on the other hand, adsorb slowly to strain U95.

Properties of T6·U95: In order to test whether adsorption of T6·U95 to strain BB was normal, a logarithmically growing culture (5×10^8/ml) was infected at a multiplicity of about 3 with radioactive phage. At intervals, samples were removed and the number of adsorbed phage determined by filtering onto membrane filters. Since the bacteria are retained by the filter and free phage pass through, adsorption can be measured by determining the amount of radioactivity which is retained on the filter. The rate and amount of adsorption of T6·U95 and T6·HfrC to strain BB were similar. In a control experiment, no adsorption of these phages to strain S/6 was detected. Thus, the modification of T6·U95 does not affect adsorption to the nonpermissive host.

Upon testing the viability of cells which have been infected with T6·U95, it was found that these phages kill bacteria in which they are unable to multiply. These experiments further revealed that the apparent titer of a stock of T6·U95 increases with increasing multiplicity of infection and is also, to some extent, dependent upon the physiological state of the infected bacteria: the restriction of T6·U95 multiplication is more pronounced during the exponential than during the stationary phase of bacterial growth.

It has been shown by Dussoix and Arber[5] that a host-induced modification of phage λ results in the destruction of its DNA after infection into a nonpermissive host. To test whether a similar destruction occurs in BB infected with T6·U95, samples from cultures infected with DNA-labeled phage were withdrawn, chilled, and precipitated with cold TCA (5 per cent). These samples were filtered, washed with cold TCA, and the precipitated radioactivity was determined. No loss of counts (sufficient to account for the low EOP) was detected, an indication that there is no extensive destruction of this DNA, although degradation to acid-precipitable fragments cannot be excluded.

Glucosylation: It has been shown that the hydroxymethylcytosine (HMC) residues of T-even phage are substituted with one (or two) glucose molecules.[14–17] The pattern of glucosylation is specific for each phage type. Furthermore, it is known that the glucosylation of these pyrimidines proceeds by a transfer of glucose from UDPG to the hydroxymethyl group of HMC in DNA.[10, 18] Since strain U95 appears to be defective in UDPG biosynthesis, we have investigated whether the DNA of T6·U95 contains glucosylated or unglucosylated DNA. DNA from purified stocks of T6·HfrC and T6·U95 was prepared. Equal amounts of DNA were assayed for hexose content by means of the anthrone reaction.[19] The results of this experiment indicated that the hexose content for T6·U95 DNA is about 2 per cent that of T6·HfrC. This is only a rough estimate, and the actual glucose content in T6·U95 may actually be higher or lower.

We have tested the capacity of these DNA preparations to accept glucose in the *in vitro* glucosylating system described by Kornberg.[10] It was found that no glucose could be added to the DNA of T4·HfrC or T6·HfrC. In contrast, the DNA derived from T4·U95 did accept glucose, as illustrated in Figure 2. In these experiments, carried out most extensively with the T4 glucosylating system, it was found that about 90 per cent of the theoretically available residues could be substituted. Thus, in T6, 75 per cent of the HMC bases are normally glucosylated, and we have found that 63 per cent of the HMC residues can be substituted using DNA from T6·U95. In T4, 100 per cent of the HMC is normally glucosylated; using T4·U95 DNA we found that 80–100 per cent of the HMC residues can be glucosylated by either the α- or the β- T4 glucosyl transferase. Similar results have been obtained by Josse and Kornberg using HMC-DNA synthesized *in vitro*.[20]

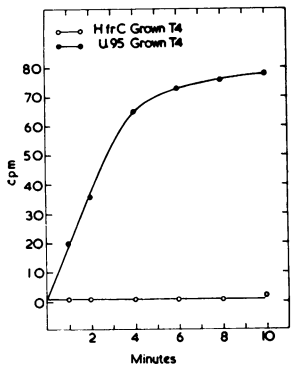

FIG. 2.—Glucosylation of DNA from T4·HfrC and T4·U95. The experimental procedure is presented under *Materials and Methods*.

Discussion.—These experiments demonstrate that the DNA of T-even phages which have been propagated on strain U95 contains little or no glucose. Such phages will form plaques with relatively high efficiency on U95 or HfrC, yielding what is apparently normal T6. The presence or absence of glucose does not affect the nature of the genetic information contained in the DNA, but only the capacity of the phage to grow in certain hosts. On strain B, growth of nonglucosylated phage is restricted, and this might be due to the presence in this bacterial host of an enzyme which degraded such DNA. We have been unable, however, to demonstrate extensive degradation of the DNA such as was found by Dussoix and Arber[5] for host-modified λ. If the restricting enzyme makes only a few breaks in the modified DNA, our failure to find extensive degradation may arise from the resistance of HMC-DNA to subsequent hydrolysis by other enzymes which attack cytosine-DNA. The presence of glucose on the HMC bases might inhibit the action of the restricting enzyme. There may be many such restricting enzymes with different specificities, and the varying patterns of glucosylation in the different T phages may reflect the range of such specificities. For example, although T2 is not restricted on strain B despite the fact that only 75 per cent of its HMC bases are glucosylated, other bacterial

strains may exist which restrict T2, but not T4, which is fully glucosylated. In fact, *E. coli* K-12 may be one such strain since T2 grows with low efficiency in this host.[21]

In a reinvestigation of the T* modification, Hattman and Fukasawa[22] have shown that the modifying host, strain $B/4_0$, is defective in UDPG pyrophosphorylase, and that strain W4597, also defective in this enzyme,[6] produces similarly modified phage. Revertants of strain $B/4_0$ not only regain enzyme activity, but also lose the ability to modify T2 and T6. Thus, it appears that the three mutants, $B/4_0$, W4597, and U95, produce a class of phage modifications of T-even phage due to unglucosylated HMC-DNA. Whether strain U95 has the same genetic defect as $B/4_0$ and W4597 has not yet been ascertained; nor has it been tested whether the phages grown on these various bacteria behave identically with respect to host range.

In view of the recent demonstrations[23, 24] that DNA may contain bases other than cytosine, thymine, adenine, and guanine, it seems likely that the chemical basis for host-induced modification, in general, may be analogous to that described here. For example, host-controlled variations in methylation of certain bases could lead to the production of biologically modified viruses.

Summary.—A mutant of *E. coli* HfrC which is defective in UDPG biosynthesis has been shown to modify T-even phage. The biological expression of this modification consists of the failure to grow in derivatives of *E. coli* strain B. A chemical basis for this modification has been found in that the DNA of these phages contains little or no glucose.

* Postdoctoral fellow of The National Foundation.

[1] Luria, S. E., and M. L. Human, *J. Bacteriol.*, **64**, 557 (1952).

[2] Luria, S. E., in *Viruses*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 18 (1953), p. 237.

[3] Christensen, J. R., *Virology*, **16**, 133 (1962).

[4] Arber, W., and D. Dussoix, *J. Mol. Biol.*, **5**, 18 (1962).

[5] Dussoix, D., and W. Arber, *J. Mol. Biol.*, **5**, 37 (1962).

[6] Fukasawa, T., K. Jokura, and K. Kurahashi, *Biochem. Biophys. Res. Comm.*, **7**, 121 (1962).

[7] Kurahashi, K., and A. J. Wahba, *Biochim. Biophys. Acta*, **30**, 298 (1958).

[8] Brenner, S., unpublished.

[9] Adams, M. H., *Bacteriophages* (New York: Interscience, 1959).

[10] Kornberg, S. R., S. B. Zimmerman, and A. Kornberg, *J. Biol. Chem.*, **236**, 1487 (1961).

[11] Allen, R. J. L., *Biochem. J.*, **34**, 358 (1940).

[12] Shedlovsky, A. E., H. A. Boye, and S. Brenner, in preparation.

[13] Wiesmeyer, H., and E. Jordan, *Anal. Biochem.*, **2**, 281 (1961).

[14] Jesaitis, M. A., *Microbial Genetics Bull.*, **10**, 16 (1954).

[15] Sinsheimer, R. L., *Science*, **120**, 551 (1954).

[16] Volkin, E. J., *J. Am. Chem. Soc.*, **76**, 5892 (1954).

[17] Lichtenstein, J., and S. S. Cohen, *J. Biol. Chem.*, **235**, 1134 (1960).

[18] Kornberg, A., S. B. Zimmerman, S. R. Kornberg, and J. Josse, these PROCEEDINGS, **45**, 772 (1959).

[19] Knudson, H. W., V. W. Meloche, and C. Juday, *Ind. Eng.* (Chem. Anal. ed.), **12**, 715 (1940).

[20] Josse, J., and A. Kornberg, *J. Biol. Chem.*, **237**, 1968 (1962).

[21] Streisinger, G., and J. Weigle, these PROCEEDINGS, **42**, 504 (1956).

[22] Hattman, S., and T. Fukasawa, these PROCEEDINGS, **50**, 297, (1963).

[23] Kallen, R. G., M. Simon, and J. Marmur, *J. Mol. Biol.*, **5**, 248 (1962).

[24] Dunn, D., and J. D. Smith, *Biochem. J.*, **60**, xvii (1955).